

**A STUDY OF THE EFFECTS OF CLAY EATING ON IRON
STATUS AND GROWTH RATE USING RATS.**

BY

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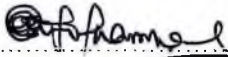
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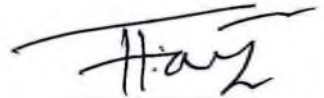
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DECLARATION

This study was undertaken by me, as presented under the supervision of Francis A.K Tayie, of the Department of Nutrition and Food Science, University of Ghana, Legon.



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Francis A.K Tayie
(SUPERVISOR)

DEDICATION

This project is dedicated to the Most Excellency, The Almighty God, for his protection, love and guidance, which made it possible for me to study at this rather difficult time of my life.

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ABSTRACT

In this study, the effects of clay ingestion on iron status and other anaemia factors were investigated using rats. Rats were fed clay at the levels consumed by Ghanaians (weight for weight) who have clay pica habit. A total of 28 white stock weanling rats, aged 3 weeks, were fed experimental diet containing clay for 11 weeks, an equivalent of four years in humans on lifespan basis. The rats were randomly assigned to four different experimental diets containing different levels of clay: a control diet, 0.00g clay/100g diet; test diet I, 0.95g clay/100g diet; test diet II, 6.43g clay/100g diet; test diet III, 1.57g clay/100g diet. Data on packed cell volume (PCV), serum iron concentration and haemoglobin level were collected. Body weights and lengths of the rats were measured weekly to assess the effect of clay ingestion on growth. Weights of essential body organs; kidney, liver, spleen and heart were also measured. Results showed that rats on the control diet had mean haemoglobin concentration, 14.4g/dL, significantly lower than those on the test diets: test diet I, 17.6g/dL ($P < 0.019$); test diet II, 17.4g/dL ($P < 0.020$); and test diet III, 16.0g/dL ($P < 0.040$). The PCV followed a similar trend, rats on the test diets had significantly higher PCV, $P < 0.05$, when compared to rats on the control diet. However, all the measured concentrations were within normal range; Serum iron concentration was highest for the rats on test diet II compared with rats on the control diet, 9.33 versus 3.29 mg Fe/100ml respectively, $P < 0.0001$. No significant differences were observed among the rats on the diet groups in terms of linear growth, body weight and appearance of essential body organs. It was concluded that the type of clay commonly ingested

in Ghana improved haemoglobin level and serum iron concentration of rats.

Thus, clay consumption was not associated with anaemia in rats.

TABLE OF CONTENTS

	<u>PAGE</u>
Title page	i
Declaration	ii
Dedication	iii
Acknowledgement	iv
Abstract	vi
Table of contents	viii
List of tables, figures and plates	xi

CHAPTER ONE: INTRODUCTION

1.1	Background information	1-2
1.2	Main objective	3
1.3	Specific objectives	3

CHAPTER TWO: LITERATURE REVIEW

2.1	Definition of pica	4
2.2	History of pica	4-5
2.3	Pica in Ghana	6-7
2.4	Mineral contents of white clay ingested in Ghana	7
2.5	Pica around the world	7-11
2.6	The impact of pica on health	12-13
2.7	Pica and iron deficiency anaemia	13-17
2.8	Reported beneficial effects of pica	17-19
2.9	Pica and infant birth-weight	19-20
2.10	Animal experimentation	20-22

2.11	Rats in laboratory investigation	22-27
2.12.1	Iron requirements and metabolism in humans	28-31
2.12.2	Distribution of iron in human body	31-32
2.12.3	Iron deficiency anaemia	32-35
2.13	Iron nutrition and the rat	36-37

CHAPTER THREE: METHODOLOGY

3.1	Study site	38
3.2	Animals used	38
3.3	Housing of the rats	38
3.4	Study design	39
3.5	Diet preparation	41
3.6	Determination of the level of clay to be fed	41
3.7	Determining the duration of feeding in relation to duration of clay ingestion by humans	43
3.8	Laboratory procedures	43
3.8.1	Weight measurement	43
3.8.2	Biochemical data	44
3.8.2.1	Blood collection	44
3.8.2.2	Haemoglobin determination	44
3.8.2.3	Serum preparation	44
3.8.2.4	Serum iron determination	45-46
3.8.2.5	Packed cell volume	46
3.8.3	Clinical examination	46

3.8.4	Determination of iron in the feed and clay samples	47
3.8.5	Wet Ashing of the clay and diet aliquots	47
3.8.6	Moisture determination	47-48
3.9	Data presentation and analysis	48
3.10	Precautionary measures	49

CHAPTER FOUR: RESULTS

4.1	Levels of clay and feed and consumption of the experimental diet	50
4.2	Characteristics of the experimental animals at the start of the study	51
4.3	The growth rate of the experimental rats	51-54
4.4	Indicators of iron status	55-56
4.5	Weights and appearance of essential body organs (kidney, liver and spleen)	56-57
4.6	Clinical symptoms	57

CHAPTER FIVE: DISCUSSIONS

5.1	Iron status	58
5.2	Haemoglobin concentrations	58-59
5.3	Growth	60
5.4	Clinical signs and effects on body organs	60-61

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1	Conclusions	62
6.2	Recommendations for future work	62 - 63
	REFERENCE	64-72
	APPENDICES	73-75

LIST OF TABLES, FIGURES AND PLATES

		<u>PAGE</u>
Table 3	Determination of serum iron concentrations of rats fed Experimental diet	45
Table 4.1	Average daily dietary intakes and proportions of clay and feed In the experimental diets of the rats	50
Table 4.2	Haemoglobin (Hb) concentration, PCV and serum iron concentration of rats fed experimental diets containing different levels of clay for 11 weeks compared with controls	55
Table 4.3	Concentration of iron (Fe) in the experimental diets consumed by rat per day	56
Table 4.4	Weights and appearance of essential body organs of rats fed experimental diets containing different clay concentration	56
Figure 1	Summary of the study design	40
Figure 2	Schematic of the experimental diet preparation	41
Figure 3	Mean weekly incremental weight gain of rats on experimental diets containing different levels of white clay	52
Figure 4	Total weight gain of rats fed different levels of white clay for 11 weeks	53
Figure 5	Growth in length of rats fed experimental diets containing Different levels of white clay for 11 weeks	54
Plate 1	Rats fed on experimental diets at the beginning of the study	49a
Plate 2	Rats fed on the clay diets for 10 weeks	54a

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND INFORMATION

Clay ingestion is a major form of pica among females, mostly pregnant women in Ghana (Tayie and Lartey, 1999; Twenefour, 1999). The term pica is derived from the Medieval Latin word for magpie, the name for a bird known for its enormous appetite for diverse substances (Walker *et al*, 1997; Glickman *et al*, 1981). Pica has been defined in several ways due to the diversity of the habit. Some authors define pica as the desire to ingest unusual things such as chalk, charcoal, wooden slate, refrigerator frost, pencil, clay and salt, (Kroger *et al*, 1962). However pica has also been described as the compulsive ingestion of non-food substances over a sustained period of time (Walker *et al*, 1997).

Clay ingestion falls under geophagia which is a sub-division of pica involving the compulsive consumption of clay and other materials from the lithosphere (Johns and Duquette, 1991; Glickman *et al*, 1995). According to Mcloughlin (1987), geophagia is the most common form of pica in the tropics.

Clay ingestion has been attributed to several reasons including its flavour and prevention of antenatal nausea and vomiting (Tayie and Lartey (1999). According to Bogert *et al* (1973), clay ingestion may be partly due to natural craving for trace minerals, especially iron.

In Ghana, white clay ingestion is the most common form of pica. People of the Akan lineage in Ghana call the white clay as "Shirew" while the people of

the Gas tribe call it "Ayelo" (Twenefour, 1999). This clay product is obtained from a raw material called "Agatawoe", mined locally from the high altitude plains of a town called Anfoega and its surrounding areas in the Volta Region of Ghana (Koduah, 2000).

Studies on clay ingestion in Ghana and other parts of the world have indicated its association with anaemia but have not shown a causative relationship (Tayie and Lartey, 1999; Gibbs and Seitchik, 1980). However, Talkington *et al* (1971) after studying two clays from Eastern Texas, which were popular with pregnant women, reported that there was a minimal impairment of iron absorption when the clays were ingested prior to iron intake. Bronstein and Dollar (1974) and Whitlam (1975) reported that correction of anaemia reduced clay ingestion. Coltman (1969) reported that pica is practiced by some patients with iron deficiency anaemia apparently to correct this deficiency. Latham (1997) also published that iron deficiency anaemia can lead to pica habit. It is obvious from these studies that the role that geophagia plays in the development of iron deficiency anaemia has been reported with conflicting views. It is unclear whether it is anaemia that induces clay pica or it is rather clay pica that causes anaemia.

The aim of this study therefore, was to investigate the effects of clay pica on iron status, body organs and growth using rats. This will enable informed advice on clay ingestion for those who practice pica, especially pregnant and lactating women in Ghana.

1.2 Main Objective

To study the effects of clay pica on iron status, growth, body organs and some haematological indices in rats.

1.3 Specific Objectives

- i. To study the effects of feeding experimental diet containing white clay on packed cell volume (PCV), haemoglobin concentration and serum iron concentrations in rats.
- ii. To study the effect of feeding white clay on the weekly weight gain of rats fed experimental diets containing different concentrations of white clay.
- iii. To study the effect of feeding white clay on the weights and appearance of essential body organs; kidney, liver, heart and spleen.

CHAPTER TWO

LITERATURE REVIEW

2.1 Definition of pica

Pica refers to the ingestion of a non-food or foods items abnormal in quantity or kind (Kroger *et al* 1962;WHO, 1961). Pica has also been described as the compulsive ingestion of non-food substances over a sustained period of time (Walker *et al*; 1997) or the pathologic craving of substances not commonly regarded as food (Lacey, 1990; Halsted, 1968).

The subdivisions of pica include; pagophagia - the excessive consumption of ice and refrigerator frost; amylophagia - the consumption of raw uncooked starchy materials; geophagia - the consumption of clay, dirt, soil and other lithosphere; plasticophagia – the chewing, suckling and nibbling of plastic materials.

2.2 History of pica

According to Danford (1982), the historical descriptions of pica began as early as 40 BC, in the days of philosophers like Aristotle and Hippocrates. These philosophers were interestingly concerned with the dangers of consuming large quantities of ice water and snow (Walker *et al*; 1997). The ancient Roman Physician, Soranus, is known for describing how pica was used to alleviate symptoms and the unpredictable appetite associated with pregnancy. He reported that pregnant women were consuming some unusual food items and

that the craving for such unusual food items began around the fortieth day of pregnancy and persisted for at least four months (Coltman, 1969). He explained that the damage from substances, which satisfy the desires in an unreasonable way, could harm the foetus as well as the mother (Coltman, 1969; Jackson, 1988).

Geophagia can be traced back to the 18th century when the sultan of Turkey consumed special clay from the island of Lemnos leading to some Europeans adopting it as a health food (Deutch, 1977). The practice of geophagia was also reported as a public health hazard in the Southern States of North America in 1825 (Mokhobo, 1986). It was believed that geophagia occurred largely through the slave trade of Africa and the Americas (Vermeer and Frate, 1979).

In Africa during the 18th to the early 20th century, explorers and missionaries reported clay ingestion in Nigeria, Ghana and Sierra-Leone (Hunter, 1993). During the early part of the 20th century, miners in Australia ate earth and mountain tallow, which were spread onto bread as a substitute for butter while in Southern Germany; quarrymen developed "Stone butter" obtained from clay (Hawass *et al*, 1987).

During the 9th Century AD, Aetius, Royal Physician, Justinian I, presented the first documented description of pregnancy-related pica (Coltman, 1969).

2.3 Pica in Ghana

Until recently, the prevalence rate of pica in Ghana was not known due to the fact that only little research has been carried out on the pica practice in the country. The major form of pica in Ghana is geophagia with the ingestion of white clay being the most common, 28% total pica prevalence (Tayie and Lartey, 1999). This white clay is known as “ayelo” by the Gas and “shirew “ by the Akans in Ghana (Twenefour, 1999). The white clay is mined in Anfoega, a town in the Volta Region of Ghana. It is processed and molded from a local raw clayey material known as “agatawe”. In the Ewe language of Ghana, “we” refers to clay and ‘Agata’ is one of the ten towns in the Anfoega area where clay processing is believed to have started over 300 years ago, hence the name “Agatowoe (Koduah, 2000). The clay is mined from the high altitude plains of Anfoega and it provides employment for most of the people of Anfoega and its surrounding areas.

Pica in Ghana is common among pregnant and lactating women (Tayie and Lartey, 1999; Twenefour, 1999). From these studies reasons given by clay consumers include; the flavour of the clay is pleasant, effects of antenatal nausea, diarrhea and vomiting (Tayie and Lartey, (1999) and relief of stomach discomfort (Bateson and Lebroy, 1978; Morgan, 1984). Among 502 pregnant women studied, 48.01% had pica habit with clay ingestion being the major form of pica (28.49%). This was followed by chewing of wooden sponge or stick (9.76%), Cola nuts (4.98%), uncooked maize dough (2.79%), chalk (1.20%) and fresh starch (0.80%) (Tayie and Lartey , (1999).

2.4 Mineral contents of the white clay ingested in Ghana

According to Koduah (2000), the mean concentrations of selected minerals from clay samples obtained from the Madina and Makola markets were (mg/100g) zinc, 44.8; iron, 838.09; magnesium, 64.58; lead, 2.36 and aluminum, 1237.27. She observed that in the order of abundance, aluminum was extracted in the highest quantity followed by iron, magnesium, zinc and lead Koduah, (2000). The accessible mineral contents of the Anfoega clay were observed to be (mg/100g) 0.9 for zinc, 10.68 for iron, 17.40 for magnesium and 37.84 for aluminum.

2.5 Pica around the world

The practice of pica is worldwide. This practice has been attributed to several reasons.

According to Bogert *et al*, (1973), this practice may result partly from natural craving for trace minerals, especially iron. Tayie and Lartey, (1999), in their study on pica practice among pregnant Ghanaians and its effects on infant birth-weight and maternal haemoglobin level, also observed that pregnant women in Ghana practice pica, more especially clay ingestion possibly due to the effects of antenatal nausea and vomiting and the other reason is probably due to its flavour.

Estimates of the prevalence of pica vary due to the difficulty involved in acquiring accurate information from patients and the lack of uniformity in the definition of terms (Glickman *et al*, 1981). It is associated with a range of

physical, environmental, social, mental and personality disturbances (Anonymous, 1992), and with psychological abnormalities and mental retardation (Garcia *et al*, 1987).

Most studies on pica in pregnancy have been confined to evaluating the ingestion of clay and dirt (geophagia), starch (amylophagia) or ice (pagophagia), (Ferguson and Keaton, 1950; Keith *et al*; 1968; Edwards *et al*; 1959; Vermeer and Frate, 1979; Coltman, 1969; Reynolds *et al*, 1968; Hook, 1978 and Mc Gannity *et al*,1969).

The most common form of pica in the tropics is geophagia (Mcloughlin 1987) and it is prevalent during pregnancy and lactation. Studies by lacey, (1990), and Danford (1982) show that in the United States, pregnant women (especially rural, Southern, and black) are considered to be at high risk for pica. The prevalent rates in pregnancy for selected study populations have ranged from 0% in urban white patients to 68% in rural black patients (Ferguson and Kaeton, 1950; Keith *et al*, 1968).

According to Ethel *et al*,(1978), pica is mainly practiced in poorly fed individuals in the lower socio – economic group in the developing countries.

Reports by Mcloughin (1987); Horner *et al*, (1991) indicate that in America, geophagia is more common among Afro-American women and those living in rural areas.

In rural Holmes country, Mississippi, it has been reported that geophagia is common stemming from deeply imbedded cultural traditions and attitudes. In

that community, the practice prevailed in 57% of the women. The average daily clay intake by the women was approximately 50g.

In Jamaica, a study on the dietary habits of rural women during pregnancy revealed that 15 out of the 38 pregnant women used for the study reported cravings. The common cravings include the following: stone (20%), cigarette ash (13.3%) and drinking soda (13.3%). Cravings for marl, raw oats, green mango, baby powder, sulphur and ash were also reported (Melville and Francis, 1992). The women gave reasons such as "I feel like eating it and I get to like it".

Clay ingestion has spread into rural areas and cities where it is at times obtained from supermarkets or, alternatively, mailed from home (Twenefour, 1999). According to Roselle (1970); Crosby, 1976a when clay is unavailable, laundry powder is sometimes used as a substitute. According to Geissler *et al* (1998), 38 pregnant women in Kenya who reported eating soil regularly were interviewed on geophagy and it was observed that the most commonly eaten soil was from the walls of houses.

The median estimated daily soil intake was 41.5g (range 2.5 –219.0g). When the soil samples were analyzed for their iron, zinc and aluminium contents, the average daily intake supplied the geophagous women with 4.3mg of iron, corresponding to 14% of the recommended dietary allowance of iron for pregnant women.

Geissler *et al*, (1998) also studied iron status and anemia among 156 primary school children of median age 13 years in Western Kenya. Geophagia was assessed through interviews and 114 (73.1%) of these children reported

eating soil daily. Analysis of the soil eaten by the children revealed a HCl – extractable iron content of 168 – 169mg/kg (SD 44.9). Based on the data on the amounts eaten daily. This means that iron content in soil could provide on the average, 4.7mg iron to a geophagous child. This is equivalent to 32% of the recommended nutrient intake (RNI) for girls or 42% of the RNI for boys.

A study by Grigsby *et al* (1999), revealed that the ingestion of kaolin, also known as white dirt, chalk or white clay is a relatively common type of pica found in the central Georgia Piedmont area. Studies have indicated that although geophagia has been observed and documented in many areas of the world, the specific preference for consuming kaolin is less well known (Grigsby *et al*, 1999). From their study, kaolin ingestion in Central Georgia appears to be a culturally-transmitted form of pica, not selectively associated with other psycho-pathology.

The practice of pica is widespread in Africa. This is variously associated with medicinal treatment and with spiritual ceremonial behaviors (Twenefour, 1999). Pica practice has been observed in Nigeria, Ghana and Sierra-leone (Hunter, 1993). Clay ingestion has also been reported to be widespread among pregnant women in five African Countries namely; Zambia, Swaziland, Malawi, South Africa and Zimbabwe and an estimated prevalence of 90% has been reported in rural areas (Walker *et al*, 1997).

In Africa, some influential factors affecting pica practice are social habit of geographical origin, age (children), race, poverty, starvation, folk medicine and social custom, neurosis, mental aberration, periods of life when there are increased requirements for nutrients (growth, pregnancy, lactation) and ethnic-

cultural response to pregnancy and lactation and also superstition and emotional disturbances (Mokhobo,1986).

In South Africa, the frequency of pica among urban and rural African women was reported to be 38.3% and 44.0% respectively (Walker *et al*, 1985).

In Malawi, it is reported to be surprising for a pregnant woman not to practice pica since it is their way of identifying if she is really pregnant. The taste of clay is claimed to diminish the nausea, discomfort and vomiting in “morning sickness” during pregnancy. Clay ingestion in this case is seen as normal during pregnancy and not between pregnancies (Hunter, 1993). Hawass *et al*, (1987) also report that poverty, starvation and famine are some of the reasons associated with the pica practice in which case the substances function as a bulking agent to supplement insufficient food or a poor diet (Mclouglin, 1987).

In a Shona village, north of Harare, Zimbabwe, it was reported that village women variously and interchangeably consume at least six different types of locally available clay. In this connection, a premium is placed upon “Smoothness” and on absence of “grit”. Often smoked over a fire, clays are provided and kept in the house in a twist of plastic (Hunter, 1993).

Recent studies worldwide have suggested a decreasing prevalence of pica practice since the 1950s (Horner *et al*, 1991).

2.6 The impact of pica on health

Some researchers have reported that clay pica may be harmful to the consumer, while others claim that clay ingestion may be beneficial to the consumer.

According to Danford (1982), Barton *et al*, (1992); Campbell and Davidson (1970), pica is considered to be potentially harmful in pregnancy. This is based on reports of foetal and maternal toxicity, maternal nutritional abnormalities, and other maternal complications due to ingestion of certain substances in clay.

Research has shown that the microbial content of clay is high and can increase microbial load in the body (Smulian *et al*, (1995). They reported that people who eat clay might be at risk of lead toxicity or other environmental toxin exposure. When a higher amount of clay is consumed, it might occupy a substantial volume of the stomach possibly preventing adequate nutrition during pregnancy. It may also form chelates with essential minerals such as iron, copper, zinc and calcium and reduce their availability in the gut and it can also cause obstruction in the colon (Key *et al*, (1982). This condition may aggravate the malnutrition problems which occur during pregnancy especially anaemia (Tayie and Lartey, 1999).

Horner *et al* (1991) after studying pica practices among pregnant African-American women concluded that pica practices in pregnancy were more prevalent than commonly believed and may have serious side effects for both mother and infant.

A study by Federman *et al* (1997) revealed that pica is often associated with lead poisoning, abdominal problems, hypokalemia, phosphorous intoxication and dental injury. Geissler *et al* (1998) studied geophagy and its impact on geohelminth infection among 204 primary school children in Western Kenya. In this study, 77% of the children were found to eat soil daily and 48% of all soil

samples were contaminated with eggs of round worms-*Ascaris Lumbricoides*. It was concluded from the study that geophagia is likely to be a source of ascariasis and possibly trichiuriasis among primary school children.

A study has shown that those who eat ice risk fracture of teeth, a risk which is not well documented in medical literature (Rainville, 1998).

2.7 Pica and iron deficiency Anaemia

Many reports have linked pica with anaemia as either a cause or effect of anaemia (Horner *et al*, 1991 and Federman *et al*, 1997), but the evidence in either direction is inconclusive (Mitchell, 1997). From a study by Tayie and Larrey (1999), it was realized that pregnant women who ate clay tended to be anaemic and clay pica was shown to be associated with low haemoglobin concentrations; but there was no association with infant birth-weight.

Gibbs and Seitchick (1980) reported that clay ingestion seen occasionally in pregnant women is associated with iron deficiency anaemia but whether or not the ingestion of clay in large amount is the result of, or causes this anaemia is unclear.

In a study conducted on 156 primary school children by Geissler *et al*, (1998) in Western Kenya, the proportion of anaemia and iron depleted children was significantly higher among those with geophagia than those without.

Talkington *et al* (1971) reported in their study that the ingestion of starch might contribute to the development of iron deficiency during pregnancy not because iron absorption is impaired but because starch provides energy without

iron. The theory is supported by some studies, which report a rapid resolution of the eating disorder following iron therapy (Korman, 1990).

In Washington DC, a study was conducted on 553 African-American women who were admitted to prenatal clinics and it was reported that large quantity of ice and freezer frost (pagophagia), were consumed by the women resulting in serum ferritin levels of the pregnant women becoming significantly lower during the second and third trimesters of pregnancy; the average values for three trimesters of pregnancy for both ferritin and mean corpuscular haemoglobin were lower in pica women than their non-pica counterparts (Edwards *et al*, 1994). In this study, the findings were well correlated with that of more recent ones by the likes of Geissler *et al* (1998) and Tayie and Lartey, (1999).

Crosby (1976b) citing the study of Coltman (1969), proposed that pica is a symptom of iron deficiency. He estimated that more than 50% of patients with iron deficiency, practise pica of one kind or another, usually ice or food pica (Crosby, 1976a; Crosby 1976b). However, clinical studies have not been able to ascertain whether geophagy is the cause, or the result of particular nutrient deficiencies, especially iron deficiency (Johns and Duquette, 1991).

Krengel and Geyser (1978) recorded a case of a woman in South Africa who consumes 400ml of soil "almost everyday" in the morning. This woman complains of menorrhagia, which may have resulted from chronic iron deficiency.

A study on pica and iron deficiency has been reported from Reunion, Madagascar. Sixteen African girls were studied and 15 were found to have anaemia and / or iron deficiency. Blood loss was considered as a possible cause

in all patients. Of the patients, 13 ingested large amount of raw rice, 11 ingested ice cubes and 10 ingested both. They were all treated with adequate amounts of iron and pica disappeared within one or two months of treatment in all but one of the patients; biochemical evidence of iron deficiency persisted in some patients (Guidicelli and Combes 1992).

If pica interfered with the absorption of minerals or displaced iron-rich foods in the mother's diet, anaemia might be the result (Rainville, 1998). A study of pica in pregnant women in Houston and Prairie View, Texas was conducted by Rainville (1998) to determine if these practices are associated with maternal haemoglobin levels at delivery or with infant birth-weight or preterm birth. Her findings suggested that pica practices are associated with significantly lower maternal haemoglobin levels at delivery but are not associated with pregnancy outcomes. She then advised Dietitians to ask pregnant women with anaemia about pica and to counsel women who report pica regarding the health risk associated with it. In addition she found that women who practise ice pica had the highest prevalence of anaemia during pregnancy than the women who do not practice pica, (Rainville, 1998).

Takington *et al* (1971) after studying two clays from Eastern Texas, which were popular with pregnant women, reported that there was a minimal impairment of iron absorption when clays were ingested prior to iron intake. Bronstein and Dollar, (1974) and Whitlan, (1975) reported that the correction of anaemia reduces clay ingestion.

Coltman (1969) reported that pica is practical by some patients with iron deficiency apparently to correct this deficiency. Latham (1997) also reported that iron-deficiency anaemia could lead to the abnormal consumption of earth, clay or other substances. Geissler *et al*, (1998) studied geophagy, iron status and anaemia among pregnant women on the coast of Kenya. The study revealed a strong negative association between geophagy and both haemoglobin and ferritin status. At the same time, it demonstrated the potential of soil as a source of dietary iron for geophagous women. According to them, the seemingly contradictory results might be due to other components in the soil interfering with iron uptake or metabolism. Alternatively, it may be that the geophagous women had extremely depleted iron stores before starting to eat soil. Iron depletion and anaemia are associated with geophagy (Geissler *et al*, 1998).

Talkington *et al*, (1971) examined iron absorption in pregnant women who ingested laundry starch or various clays and found no delays in uptake. However, different types of clay have been shown to have different effects on the absorption of iron (Minnich *et al*, 1969).

Support for iron deficiency as a cause of pica in pregnancy, comes from studies in which the administration of iron corrects anaemia and is followed by the resolution of the particular pica (Keith *et al*, 1968 and Bronstein and Dollar, 1974).

The role that clay and other pica plays in the genesis of iron-deficiency anaemia has been reported with conflicting views as it is really unclear from

these studies whether it is anaemia that causes clay ingestion or it is the clay ingestion that causes anaemia.

2.8 Reported beneficial effects of pica

Even though research has shown that the consumption of pica may be harmful to the human health, other researchers have a different view. According to them, so many benefits can be derived from its consumption.

In a study conducted by Smulian *et al*, (1995) on pica in a rural obstetric population, patients with pica were specifically asked whether their practice was beneficial or harmful. Out of the 18 women, 3 (16.7%) of them believed it was beneficial, 3 (16.7%) also thought it was harmful, 5 (27.8%) thought it was neither beneficial nor harmful and the remaining 7 women (38.9%) were not sure.

According to Wiaz (1997), the eating of some clay has been used as a remedy for diarrhoea and stomach discomfort probably due to the presence of Kaolin in these clays and also due to its absorptive ability (Bateson and Lebroy; 1978; Morgan, 1984).

A report by Hunter (1993) indicates that clays could absolve dietary and bacterial toxins, which are associated with gastrointestinal disturbances often encountered in pregnancy. Hunter (1993) was convinced that geophagia could play a useful role and could be appreciated as a normal human behaviour.

Morgan (1984) reported that in the third world countries, where the diet may be insufficient, clay has been eaten for satiety.

The practice of geophagia especially clay, is strongly connected to folk medicine, social customs and obsessive – compulsive behaviour (Crosby, 1976). In some developing countries, clay is eaten to “line the stomach” before eating yam or fish, which may be poisonous, in order to reduce hunger and treat hook worms infestation (Bateson and Lebroy, (1978).

According to Hunter (1993), the types of clay ingested in most countries could contribute some minerals. He reported that the consumption of 40-100g of white copper belt clay per day contributed 15mg calcium, 48mg iron, 42mg Zinc and small amounts of copper, chromium, nickel and molybdenum. These amounts according to him, are nutritionally significant where dietary deficiencies exist. He further stated that for women, the white clay at a consumption rate of 100g per day would supply 322% of the Recommended Dietary Allowance (RDA) for iron 70% for copper and 43% for manganese.

A publication by Tayie and Lartey (1999) revealed that some pregnant women in Ghana consume clay because according to them, it prevents vomiting and antenatal nausea and even anaemia. In the Southern States of the United States of America, pregnant women who traditionally ate substances like clay, corn, starch and baking soda, had the belief that such substances helped to prevent vomiting, helped babies to thrive, cured swollen legs and ensured beautiful children (Twenefour, 1999).

In Australia, the eating of clay by some Aborigines is due mainly to medicinal purposes. (Bateson and Lebroy, 1978). In a study by Geissler *et al*, (1998) on geophagy, iron status and anaemia among pregnant women on the

coast of Kenya, demonstrated the potential of soil as a source of dietary iron for geophagous women. The average daily soil intake supplied the geophagous women with 4.3mg of iron, which corresponds to 14% of the recommended dietary allowance (RDA) of iron for pregnant women.

In another study by Geissler *et al*, (1998) on geophagia, iron status and anaemia among 156 primary school children in Western Kenya, the soil eaten by the children was analysed and found to contain a mean HCl – extractable iron content of 168.9mg/kg. Based on the amounts of clay eaten daily and the mean iron content, soil could provide an average of 4.7mg iron to a geophagous child which corresponds to 32% of the Recommended Nutrient Intake (RNI) for girls or 42% for boys.

Indeed, pica will remain an attractive subject, in which our knowledge and understanding are far from complete.

2.9 Pica and infant birth-weight

Most reported studies on pica did not associate it with infant birth-weight. In a study on pica among pregnant Ghanaians and its relationship with infant birth-weight and maternal haemoglobin level, no significant association was found between geophagia and infant birth-weight at the level of intake in Ghana.

Rainville, (1998) in a study on the association between pica practices of pregnant women and lower maternal haemoglobin level at delivery, classified 286 women who were visiting one of four WIC clinic waiting rooms in Houston or Prairie View, Texas, into four different groups namely; ice pica group, ice/freezer

frost pica, other pica and no pica. Her study showed no association between pica and birth-weight or gestational age. There were no significant differences between mean birth-weight or mean gestational age for the 4 groups. There was also no difference in the prevalence of low birth-weight infants among African-American women who practiced pica compared to those who did not. Even though studies are yet to show any clear association between pica and infant birth-weight, there had been reports of fetal and maternal toxicity due to the ingestion of certain substances regarded as pica (Danford, 1982; Barton *et al*, 1992; Pearl and Boxt, 1980).

2.10 Animal experimentation

Research with human beings is strictly regulated because of various ethical considerations which have come up for sometime. Hence animal experimentation has been used in several nutrition researches.

The World Federation of Animal Welfare, United Kingdom, among others, have tried to ensure that animals used in the laboratory are kept under good conditions and are not subjected to procedures that can cause undue pain to them. These groups visit research laboratories to ensure that certain standards are maintained.

A published report showed that some people or investigators were abusing the use of animals in experimental research around late 1980s. A conference was therefore called in England on; Animal Experimentation and Future of Medical Research. Among the several issues that came up and the points that were agreed on are the following;

i. Continued research with animals is still essential for the consequence of medical problems such as HIV/AIDS as well as certain psychiatric conditions.

ii. Such animal studies should be supported if they will benefit society and if there are no alternative methods and all reasonable steps are taken to avoid unnecessary pain and suffering to animals. Even in the United Kingdom, legislation already exists that permits work on animals only by people with license (Owusu, Personal communication).

Several advantages of working with the animals rather than humans have been outlined. Some of these include the following;

i. They are less restrictive regulation.

ii. The use of large numbers of animals at a time is possible which makes favours statistical analyses and experimental design.

iii. Highly purified diet of known composition can be fed in animal studies and it is possible without being too unethical to vary the intake of single nutrient as a way of investigating the effect of their deficiencies.

Others include, the possibility of studying nutritional deficiencies in relation to disease, decreased growth, longevity and reproduction using animals and it is easier to undertake biochemical studies. Animals commonly used in experiments include the rat, mouse, hamster, guinea pig, rabbits, dogs, chicken, and monkey.

2.11 Rats in laboratory investigations

The rat is omnivorous and resists many diseases. Studies have shown that the rat has dietary requirements similar to humans. It has negligible preference. Due to its small size, the cost of feeding is low and it takes little space to keep them. It has short life span of 3 – 4 years (Grindeland, 1996) so it is easy to study them. Their rapid growth rate also allows them to give results very quickly, They have sexual maturation in only 50 days and the gestation is 21 days with a very short oestrus cycle of 5 – 6 days. They can reproduce up to 5 times in a year (Owusu, Personal communication). The lactation period is critical to the rats since unlike humans, they can not bottle feed.

Despite the fact that the rat is resistant to diseases, it is also susceptible to contracting various diseases. Diseases such as Myoplasma can attack it and they are also susceptible to lice infestations especially in their hair. Others such as streptococcus pneumonia may affect their lungs and kill within 1 – 2 weeks. They may also be infested by roundworms (Owusu, Personal communication).

Rats can also be infested by tapeworm and this can produce larvae that hide in the liver and gradually cause malnutrition. Mammary tumour can spread to the lungs and liver and eventually kill the rat.

In the animal room, where the rats are kept, odour, moisture, humidity, dust, bacteria, temperature, noise level and human traffic should be controlled as well as light and ventilation. Studies have shown that proper ventilation keeps the concentrations of dust and bacteria low. The rats, however, deliberately generate some body odour from some preputial gland, which makes them feel at

home in their quarters. If the relative humidity is too low, it must be raised. Alternation of light and dark periods especially when the experiment involves reproduction is essential. For breeding, a rat needs about 12 – 16 hrs of light and 8 – 12 hrs of darkness everyday. Rats sleep in the daytime while they eat mostly at night (Owusu, Personal communication).

Several nutritional studies have been carried out using rats. The effects of salts (sodium chloride) supplementation of rat diets (80g/kg diet) with or without lactose (150g/kg diets) were studied in weanling rats over 14 days. It was detected that dietary salt increased water intake and reduced weight gain and food conversion efficiency, but the variables were unaffected by lactose. Salt – supplemented rats exhibited a 3-5-fold increase in urinary calcium excretion and a small increase in urinary magnesium and phosphorus excretion, irrespective of dietary lactose content. In addition, salt supplementation reduced plasma alkaline phosphate activity (Shortt and Flynn, 1990).

Susan *et al*, (1990) reported on the apparent absorption of zinc by rat from foods labeled intrinsically and extrinsically with zinc. In the study, a variety of foods enriched with the stable isotope zinc were prepared by means of intrinsic and extrinsic – labeling procedures and fed to rats. The apparent absorption of ⁶⁷ Zn determined from faecal excretion measurements using thermal ionization mass spectrometer. There were significant differences in the absorption of the extrinsic and the intrinsic label, which differed in magnitude between the foods tested. The study indicated that extrinsically – added stable zinc isotopes do not

fully exchange with endogenous zinc in many foods and illustrated the need for caution when using extrinsic labels for zinc bioavailability study.

In another study by Anantharaman-Barr *et al*, (1990), it was reported that the rats' oestrous cycle had little effect on CHO (Sucrose) intake. The study indicated that the hypothesis that serotonergic mechanisms play a role in the effect of oestrogen on appetite was therefore not supported. In present work, they examined the effects of the oestrous cycle on fat and protein selection where 8 Wistar rats were offered a dual choice of milled chow (metabolizable energy (ME) 13.4kJ/g) and beef fat (lard) for at least four consecutive cycles and subsequently offered a choice between chow and protein (granulated casein). The results obtained from this with those of the previous study, suggest that anorexia associated with high oestrogen levels in rats and that pro-oestrus is not the reason for the aversion to any of the 3 major macro-nutrients.

Kawabata *et al*, (1989) suggested that iron loading of mice stimulated lipid peroxidation and oxidation of glutamine. This study investigated the effects of iron on antioxidant enzymes in rats. Two groups (n=6) of female and male weanling Sprague – Dawley rats were housed individually and fed ad libitum on synthetic diets containing 15mg or 400 mg of Fe/kg. Haemoglobin, transferrin saturation and hepatic iron were measured as indices of iron status. The result of the study indicated that iron status was significantly higher in rats fed the high iron diets and iron status was significantly higher in females than in males. Female rats also had significantly higher CPL and Lower GST and CAT activities than males. The study indicated that although some antioxidants and related

enzymes were significantly decreased in the rats fed on the higher iron diet, there were no significant differences in hepatic MDA levels.

A study by Nelson and Evans (1947) reported by Friggens *et al* (1991) also investigated potential interactions between feed protein, carbohydrate and fat on lactational performance in rats. A strong interaction between the effects of feed protein, carbohydrate and fat content on lactational performance has been shown (Friggens *et al*, 1991). The study examined the severity of that interaction whilst eliminating the possible confounding between nutrient: nutrient: energy interactions. The study showed the importance of maternal body reserves for lactation but leaves open the question of the origins of the variation between rats. Nora *et al*, (1992) reported on maternal iron-deficiency effects on a peritoneal macrophage and peritoneal natural – killer-cell cytotoxicity in rat pups. Here, cytotoxicity of peritoneal macrophages (pMs) and peritoneal natural killer (pNk) cells toward xenogeric tumor cells were studied in anaemic, suckling rats. Dams were fed 6, 12 or 200mg Fe/kg diet ad libitum throughout gestation and lactation. Pups were injected intra-peritoneally with 10^5 plaque-forming units of virus. Four (4) days later cytotoxicity of pms and pNk cells against YAC-1 mouse lymphoma cell was measured. Body weight, haemoglobin, height and viable cells yield of pups decreased significantly with decreasing dietary iron. pM cytotoxicity was significantly impaired in anaemic pups at pM-target-cell ratio.

The inhibitoion effect of vitamin D-stimulated intestinal calcium transport in rats after continuous oral administration of cadmium was studied by Ando *et al*,

(1981).. It was observed from the study that cadmium has an inhibitory effect on intestinal calcium transport stimulated by vitamin D in rats.

Shiguary *et al* (1994) in their study, hypothesized that the impaired Cu status seen after Fe loading is due either to diminishing Cu absorption followed by a decrease in biliary Cu excretion or to enhanced biliary Cu excretion with an increase in copper absorption as a secondary feature. The two possibilities were examined in a 6-week trial using rats given Cu-adequate diets containing low, normal or high amounts of Fe. It was concluded from the data that increased Fe intakes depressed Cu absorption resulting in a decrease in plasma and organ Cu concentration. As a result, biliary Cu excretion is lowered and this contributes to achieving Cu balance at high Fe intakes. Because the concentrations of Cu in plasma and bile, and also plasma ceruloplasmin (EC.1.16.3.1) activities, showed much greater % reduction with increasing Fe intake than did the concentration of Cu in organs. It is possible that increased Fe status interfered with the mobilization of Cu store.

A study on the effects of protein nutrition on the m-RNA content of insulin-like growth factor-binding protein-1 in liver and kidney of rats was carried out by Asako *et al*, (1993). They looked at the effect of quantity and nutritional quality of dietary proteins on the content of m-RNA of insulin-like growth factor-binding protein –1 (IGFBP-1) in the rat liver and kidney. IGFBP-1 m-RNA content per unit RNA increased in liver and kidney of rats fed on the protein-free diet and in those of fasted rats compared to those rats fed on a casein diet. The effect of dietary octacosanol, a long-chain alcohol, on lipid metabolism was investigated in

rats fed on high-fat diet for 20 days by Kato *et al*, (1995). The addition of octacosanol (10g/kg diet) to the high-fat diet led to a significant reduction ($p<0.05$) in the perirenal adipose tissue weight without a decrease in the cell number, suggesting that octacosanol may suppress lipid accumulation in the tissue. However, no effect was seen in the epididymal adipose tissue weight and in the lipid content of the liver. The results indicated that lipid absorption was not affected by the inclusion of octacosanol. Thus, the present results suggest that the incorporation of octacosanol into a high-fat diet affects some aspects of lipid metabolism.

As indicated by Greene (1935) one is constantly impressed with the close structural similarity of the rat to man. Even in many instances, relationships, which occur as variation in man, are found to be a usual condition in the rat, hence, the use of rat in numerous nutritional research related to humans.

2.12.1. Iron requirement and metabolism in humans

Of all the trace minerals, iron deserves the most attention. It is a problem nutrient for millions of people. Iron deficiency is a very common cause of ill health in all parts of the world, both south and north. The average iron content in a healthy adult is only about 3-4 g, yet this relatively small quantity is vital (Latham, 1997). These small but biologically indispensable amounts are related to the relative insolubility of iron and to the specialized systems required for its transport and incorporation into iron proteins; haemoglobin, myoglobin, haem

enzymes (Cytochromes), non-haem iron enzymes (flavoprotein), ferritin and hemosiderin.

Iron is found in every cell, not only of the human body but also of all living things both plants and animals. In the human body, iron is found principally in the red blood cells, mainly as a component of the oxygen carrier protein, haemoglobin. Much of the rest is present in myoglobin, a compound occurring mainly in muscles and as storage iron or ferritin mainly in the liver, spleen and bone marrow. When red blood cells die and are dismantled in the liver, the iron is retrieved and transported by iron carrier proteins back to bone marrow, where new blood cells are synthesized. Additional iron is found binding protein in the blood plasma and in respiratory enzymes. Minute quantities of iron are lost in the desquamated cells from the skin, intestine, in shed hair and nails and in the bile and other body secretions (Latham, 1997). This must be replaced by intestinal absorption of 5-10 % of the dietary iron intake of 10-30mg/day (Wintrobe *et al.*, 1981). Under normal circumstances, only about 1mg of iron is lost from the body daily by excretion into the intestines, in urines, in sweats or through loss of hair or surface epithelial cell. Iron losses are small except when blood is lost as in menstruation or haemorrhage. This implies that women's needs for iron are greater than men (14-18mg/day for women, 10mg/day for men).

It is advised that women of child - bearing age must however, replace iron lost during menstruation and childbirth and must meet the additional requirements of pregnancy and lactation. Food sources of iron for women must therefore be chosen carefully, if 2/3 of the recommended intake is to be met

within energy allowances that are not excessive. The main vital function of iron is the transfer of oxygen to various sites in the body. Haemoglobin is the pigment in the erythrocytes that carries oxygen from the lungs to the tissues. Skeletal and heart muscle (myoglobin) accepts the oxygen from the haemoglobin. Children just like the lactating and pregnant women need relatively high amounts of iron because of their rapid growth, which involves increases not only in the body but also in the blood volume.

Dietary sources of iron include variety of foods of both plant and animal origin. Foods relatively rich in iron include meat (especially liver and red meat), fish, eggs, legumes (which include a variety of beans, peas) and other pulses, green leafy vegetables, cereal grains like maize, rice and wheat contain moderate amounts of iron but because these are often staple foods and are eaten in large quantities, they provide most of the iron for many people in developing countries. Research has shown that iron-cooking pots may be a source of iron. Contrary to the notion that milk is a “perfect food”, it is a poor source of iron. Human milk contains just about 2mg of iron per litre and cow's milk only half this amount (Latham, 1997). It is important to note that the availability of iron from foods varies widely. Persons who are iron - deficient tend to absorb iron more efficiently and in greater quantities than do normal subjects. This means absorption is regulated to some extent by physiological demand. Other factors such as tannins, phosphates and phytates in food reduce iron absorption and ascorbic acid (vitamin C) rather increases its absorption. Studies have indicated that egg yolk, despite its relatively high iron content, inhibits

absorption of iron and this is not only the iron from the egg yolk it self, but also that from other foods. In general, the absorption of the haem iron in foods of animal origin (meat, fish and poultry) is usually high whereas the non-haem iron in food such as cereals, vegetables, roots and fruits are poorly absorbed.

In infants and children, the daily iron requirements are relatively high; from birth to 6 months (10mg/day); 6 months to 3 years, (15mg/day); for boys 11 - 18 years, (18mg/day), dropping to 10mg for older men while it is 18-23mg/day for girls/women aged 10-50 years and dropping to 10mg in older women (Krause and Mahan, 1979). The enrichment of breads and cereals somewhat improves women's iron intake, but iron enrichment or fortification of other foods may produce iron overload in men. Addition of iron supplements to the diet may be advisable for some women. The body carefully controls its iron stores by limiting absorption and by re -using iron derived from the break down of haemoglobin and catabolism of non-haem iron proteins. Excess iron may accumulate in the body because of the lack of an efficient excretory mechanism, and iron storage capacities may become saturated.

2.12.2. Distribution of iron in human body

In spite of the fact that iron is an abundant element in the earth's Crust, the human body contains only about 45mg/kg body weight. This iron is distributed in the body in four forms that point to its basic metabolic function (Rodwell, 1993).

A minute concentration of iron of 80 to 180µg/dl (14-32µmol/L) for men and 60-160µg/dl (11 to 29µg/L) for women is found in the plasma bound to its transport carrier protein, β-globulin, named for its function-transferrin is synthesized in the liver (Chesnut, 1992).

The greatest amount of the body's iron, about 70%, occurs in red blood cells as a vital constituent of the haem portion of haemoglobin in red blood cells. 5% of total iron is part of myoglobin and about 20% of the total body iron is stored as ferritin (protein-iron compound).

The main storage organs for iron are the liver, spleen and the bone marrow. Excess iron is stored in these body organs as haemoglobin, which interchange with ferritin as needed (Rodwell, 1993). The remaining 5% of the total body iron is distributed throughout the cells as major components of oxidative systems for production of energy.

The bioavailability of iron is dependent on its absorption, and is influenced by a number of factors. The iron enters either as haem or a non-haem iron. Absorption of iron takes place mainly in the upper portion of the small intestines and much of the iron enters the blood stream directly and not through the lymphatic system (Latham, 1997).

2.12.3. Iron Deficiency Aneamia

Literally, anaemia means "too little blood" and it is a condition in which there is a decrease in the total number of red blood cells (erythrocytes), each having a normal quantity of haemoglobin, or a diminished concentration of

haemoglobin per erythrocyte or combination of both. Iron deficiency occurs when an insufficient amount of iron is absorbed to meet the body's requirement. This may be attributed to inadequate iron intake, reduced bio - availability of dietary iron, increased need for iron (example during growth and pregnancy), chronic blood loss, lack of gastric hydrochloric acid necessary to liberate and prepare iron for absorption. Others are the presence of inhibitors of iron absorption such as phosphate or phytate or mucosal lesions that affect the absorbing surface or abnormalities in the re -cycling of iron from haemoglobin of old blood cells, inflammatory disorders or connective tissue diseases such as arthritis (NCHS, 1989). Iron deficiency and its consequent anaemia is recognized as the most prevalent specific nutrient disorder in the world today (Golden and Golden, 1981). It is estimated that over one billion people are affected worldwide (CNN News, 1993). About 90% of all anaemias are due to lack of iron. Surprisingly, anaemia occurs in developed and underdeveloped countries but mostly in the developing world where nearly 1/3 of the population is iron deficient.

Iron deficiency occurs mainly in women in their reproductive age (Wintrobe *et al*, 1981), pregnant and lactating women and is also common in children, especially those of low socioeconomic background. Iron deficiency in particular respects neither social class nor geographic situation. A woman from a comfortable background in the United States is susceptible to iron deficiency anaemia just as is the poorest villager in Africa (Rodwell, 1993).

Focusing on women of fertile age and pregnant women, their vulnerability for incurring negative iron balance is due to their increased iron needs because of menstruation and the substantial iron demands of pregnancy (Latham, 1997). In children over the age of 4 years, anaemia has been reported to occur in 0.6 to 7.7% (Pearson, 1976). Relatively, advanced iron deficiency anaemia was found in 5.5% of poor children aged 5 to 8 years. Some patients with iron deficiency anaemia have no sense of ill health. The abnormality may be discovered during the course of a medical examination for other reasons. The anaemia may develop so gradually and it interferes only moderately with work efficiency, even when the haemoglobin is as low as 9gm per 100ml. The symptoms of anaemia include weakness, fatigability, pallor, palpitation, dyspnea on exertion, dizziness and a sense of "deep tiredness" and these may be absent in even moderate to severe anaemia (less than 100g haemoglobin/L blood), whereas persons with latent iron deficiency (low serum iron, depleted iron stores but no anaemia), may complain of these symptoms (Garby, 1973)

In Sub-Saharan Africa, bioavailability of dietary iron was considered the most important determinant of anaemia in every age group except pregnant women. The impact of iron deficiency anaemia on health is particularly deleterious. It is believed to be a frequent cause of retarded growth and development, inability to work and attention deficits. The most dramatic consequences of iron deficiency anaemia are cardiac deficit and death (Taboada, 1983).

According to Latham (1997), it is interesting to note that many persons with low haemoglobin levels especially women in developing countries appear to function normally. With chronic anaemia, they have adapted to low haemoglobin level. They may indeed do reduced work, have fatigue and work more slowly, but give the appearance of performing their normal duties even though severely anaemic. According to him, severe anaemia can progress to heart failure and death.

Recent studies have also shown that, anaemia, as well as producing the symptoms and signs also leads to a reduced ability to do heavy work for long periods, to slower learning and difficulty in concentration by children in school or elsewhere and to poorer psychological development. A very important aspect of anaemia in women is that it markedly increases the risk of death of the mother during or after childbirth. The woman bleeds severely as she has low haemoglobin reserves and there is also an increased risk for her infant.

Using the criteria from the Centre for Disease Control, anaemia and iron deficiency anaemia were assessed in more than 800 inner city gravidas at entry to prenatal care. Iron deficiency was associated with significantly lower energy and iron intakes and lower mean corpuscular volume at the beginning of pregnancy. The odds of low birth-weight were tripled and of preterm delivery more than doubled with iron deficiency but were not increased in anaemias from other causes. When vaginal bleeding at or before entry to care accompanied anaemia, the odds of a preterm delivery were increased five - fold for iron deficiency anaemia and doubled for other anaemia. Inadequate pregnancy

weight gain was more prevalent among those with iron deficiency anaemia than in those with anaemia of other aetiologies (Theresa *et al*, 1992).

Surveys conducted over the past decade indicate a high prevalence of iron deficiency anaemia among preschool children and pregnant women in Brazil (Torress *et al*, 1994; Szafac *et al*, 1995 and Sau Paulo, 1998). In many parts of Brazil, more than half of the preschool children are affected; and the prevalence rate is highest in children under 2 years old. In pregnant women iron deficiency anaemia prevalence ranged between 13 and 60%. Studies (U.S DHHS/PHS, 1975-85; Yip *et al*, 1987) have demonstrated a progressive decline in the incidence of iron deficiency in both lower socioeconomic and middle class populations.

Iron deficiency anaemia, which is becoming an alarming public health problem, calls for immediate and continued intervention. One measure of fighting this condition is iron fortification and changes in lifestyle to favour adequate iron status.

2.13. Iron nutrition and the Rat

The total amount of iron in the body of the rat is quite small (about 0.005% or less) and approximately 2/3 of this is present in the blood as an essential constituent of the haemoglobin of the red blood cells. Obviously, then, an iron-poor body tends to be anaemic, and studies of the dietary requirement for iron are closely related to the studies of anaemia.

Miller *et al.* (1928) reported that the bodies of rats maintained for five weeks upon whole skim milk powder diets contain about 50% (0.0022 to 0.0025%) of the normal amount of iron (0.005%).

Rose and Hubbell (1938) have shown that young rats depleted by milk feeding to approximately 4g of haemoglobin per 100ml of blood have an iron content of about 0.0013%. Depleted animals were fed supplements of 0.00038mg of copper and either 0.001/d, 0.0038 or 0.005/mg of iron per gm of body weight per day until haemoglobin had increased to about 14gm per 100ml. Depending upon the dosage of iron given, the iron in bodies of the males average between 0.0034 and 0.0038%; the females stored on average about 12% more iron than the males when intake was the same per gram of body weight. It has been demonstrated in the anaemic rat that when inorganic iron is fed in the absence of copper, there is a marked increase in the total iron content of the liver and spleen but an appreciable increase in blood haemoglobin.

Mitchell (1997), concluded that 0.25mg of iron per day is adequate to maintain normal haemoglobin values for the rat if sufficient copper is present in the diets. High intake of iron has been shown to adversely affect copper status in ruminants (Standish *et al.*, 1969), guinea pigs (Smith and Bidlack, 1980) and rats (Bremner and Young, 1981; Bremner and Price, 1985; Johnson and Hove, 1986). Bremner and Young, (1981) suggested that intake of excess iron stimulates excretion of stored copper. If copper balance is attained after iron feeding, the suggestion of Bremner and Young (1981) implies that increased intake of dietary iron, at least as a secondary effect would enhance copper

absorption (Johnson and Hove, 1986; Johnson and Murphy, 1988; Reichmayer Lais and Kirchgessner, 1992).

There is ample evidence that young anaemic rats can regenerate haemoglobin at the rate of about 10gm in six weeks when a readily available source of about 0.25mg of iron and 0.05mg copper is furnished per day in milk-based diet. The higher requirements for reproduction and lactation are adequately met by 5mg of iron and 0.5mg Of copper per day, but these amounts may be considered more than the minimum requirements. Evans and Phillips (1939) reported that rats fed fresh milk mineralized with 1.0mg iron, 0.1mg copper and 0.1mg manganese per 100IU “remained healthy and vigorous through five generations” although reproduction difficulties were observed.

CHAPTER THREE

METHODOLOGY

3.1 Study Site

The experimental animals, rats, were housed and fed the experimental diets in the animal experimentation room but biochemical assays were done in the research laboratory all in the Department of Nutrition and Food Science, University of Ghana.

3.2 Animals used

Three week (3) old white stock weanling rats bred at the animal laboratory of the University of Ghana Medical School, Korle-Bu Teaching Hospital, were used for the study.

3.3 Housing of the rats

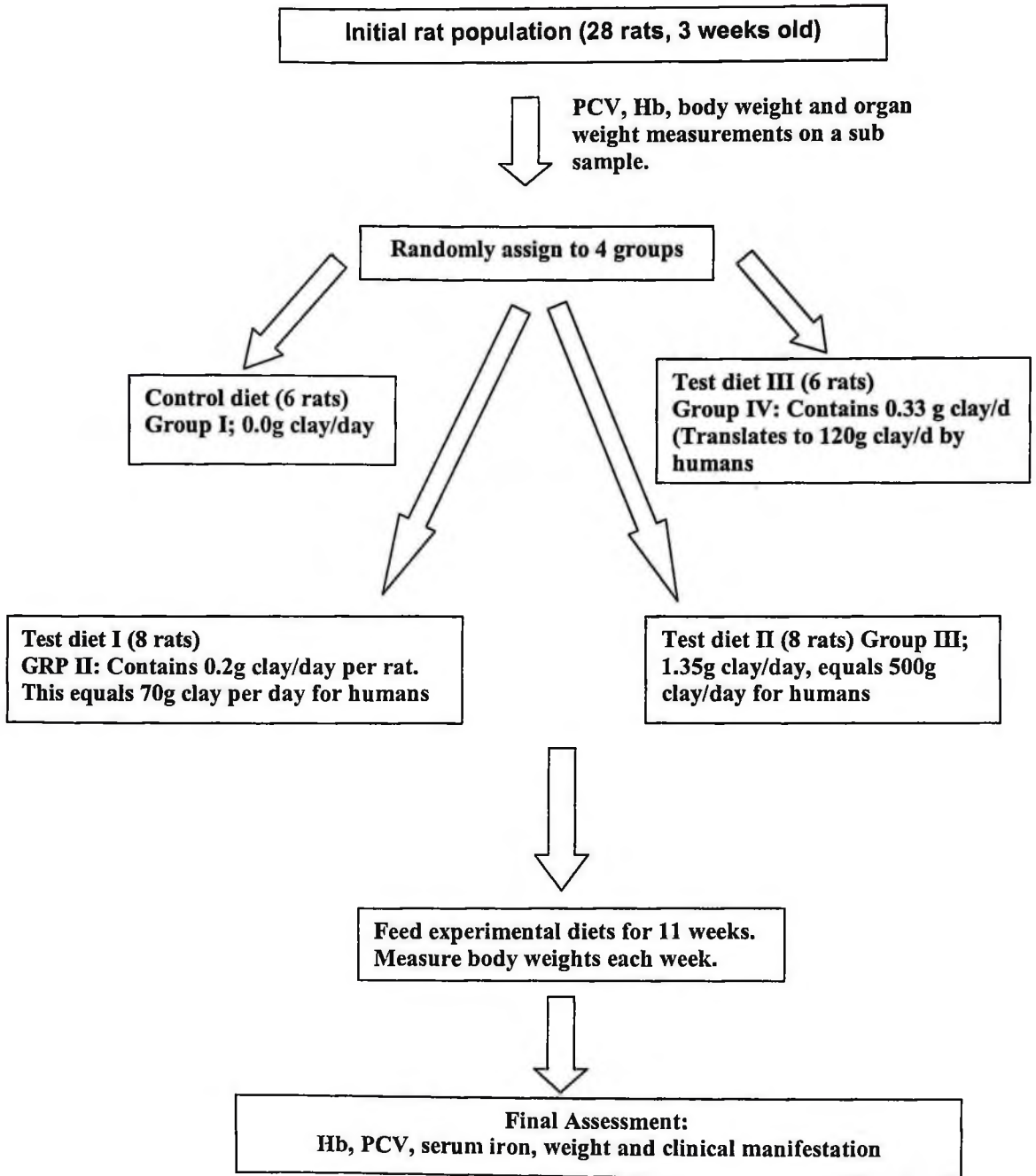
The rats were housed singly in plastic metabolic cages in the animal room of the Department of Nutrition and Food Science, University of Ghana. The room was maintained at 25-28°C with 12 h dark-light cycle. Provision was made for urine and faecal pellets collection. All stocks of diet for the study were stored in the cold room (2°C) during the study period. The rats were fed the stock diet for two weeks to enable them acclimatize to the new environment of the laboratory before the clay diet was fed.

3.4 Study design

This study was designed to simulate levels of clay ingestion observed among Ghanaian women using rats. Thirty-two white stock weanling rats from the same litter of 3 mothers were acquired from the animal laboratory of the Ghana Medical School. At the start of the study, 4 rats were randomly sampled and parameters of interest: haemoglobin level, PCV, serum iron concentration, body weight and organs weights, were measured to ascertain variation among the stock of rats. The remaining 28 rats were randomly assigned to 4 different diet groups, as shown below, and fed for 11 weeks after which final assessments were made on the listed parameters (Figure 1). The following shows how the experimental diets were allotted.

- Group I:** (6 rats) Control diet; 0.0 g clay/day per rat.
- Group II:** (8 rats) Test Diet I; 0.2 g clay/day per rat which translates to 70g clay/day for humans; weight for weight. This is the median daily clay consumption by humans (Twenefour, 1999).
- Group III:** (8 rats) Test Diet II containing 1.35 g clay/day per rat translating to 500g clay/day for humans (maximum daily consumption of clay by humans; Twenefour, 1999).
- Group IV:** (6 rats) Test Diet III containing 0.33g clay/day per rat which translates to 120 g clay/day by humans, equals the modal intake by humans.

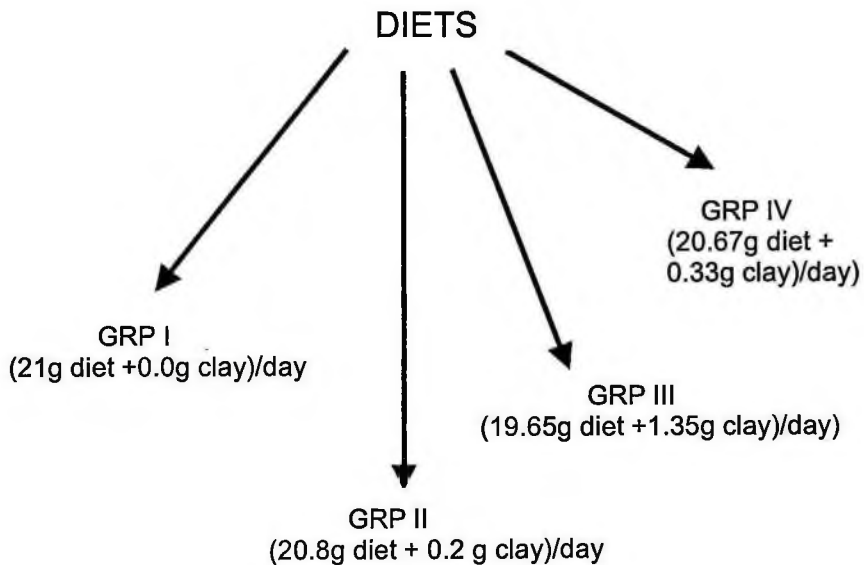
Figure 1. Summary of the study design



3.5. Diet Preparation

A Preliminary study carried out indicated that the rats on the average consumed 21g of feed per day. Portions of the feed were replaced by clay to formulate the experimental diets and fed to the rats each day for the study period.

Figure 2. Schematic of the experimental diet preparation



3.6. Determination of the level of clay to be fed

The level of clay fed was calculated per kg/body weight using an average body weight of 60kg for the Ghanaian female of reproductive age (Tayie and Lartey,

1999). Using the median 70g clay ingestion by females (Twenefour, 1999) and the observed average body weight of the rat, which was 162.46g (0.16246 kg) and a daily rat food intake of 21g feed per day, the quantity of clay to be fed per day was calculated to be 0.2g. The calculations of the amount of clay in the other diets were done similarly as shown below.

Group III

Maximum daily intake of clay by humans (upper limit) = 500g/day

Therefore for the rats, this will be equivalent to:

$$\frac{0.16246 \times 500 \text{ g}}{60}$$

$$= 1.35 \text{ g clay/day}$$

Group IV

Modal daily intake of clay by humans = 120g/day

Therefore for the rats, this will be equivalent to:

$$\frac{0.16246 \times 120 \text{ g}}{60}$$

$$= 0.33 \text{ g clay/day.}$$

3.7 Determining the duration of feeding in relation to duration of clay ingestion by humans

Average life span for humans was taken as 70 years, which is 25,550 days. Average life span for rats is 3 years, which is 1,095 days (Grindeland, 1996).

$$\begin{aligned} \text{If 70 yrs for humans} &= 1095 \text{ days for rats} \\ \text{Then feeding for 4 yrs} &= 4 \text{ yrs} \times \frac{1,095 \text{ days}}{70 \text{ yrs}} \\ &= 62.6 \text{ days} \approx 2 \text{ months for rats.} \end{aligned}$$

Thus, feeding clay to the rats for this number of days is assumed to be equivalent to feeding clay for 4 years in humans. This duration is chosen to allow for enough time for any toxic effect to manifest.

3.8 Laboratory Procedures

3.8.1 Weight measurement

The weights of the rats were measured at baseline and once every week thereafter using a sensitive Sauter scale of minimum graduation 0.01g, in the animal room of the Department of Nutrition and Food Science, University of Ghana, Legon.

3.8.2 Biochemical Data

3.8.2.1 Blood collection

Blood was drawn by venipuncture at the jugular vein while the rats were under light chloroform anaesthesia. About 5 mL of blood was collected from each of the 28 rats at the final stage for the following assessment: Haemoglobin concentration, haematocrit (PCV) and serum iron concentrations.

3.8.2.2 Haemoglobin determination

The haemoglobin concentrations were determined using a hemocue (Hemocue Inc. CA). The hemocue is a portable direct reading hemoglobinometer. Fresh blood from the rats were placed in a 10 μ L micro-cuvette, containing reagents in dried form (sodium desoxycholate to hemolyse erythrocytes, sodium nitrate to oxidize haemoglobin, sodium azide to convert methaemoglobin to methaemoglobin azide, and sodium fluorescein to standardize the light path length in the micro-cuvette). Within a minute or two, the haemoglobin value was displayed on the haemocue. This device reads absorbance at 565nm and 880nm. The absorbance read at 880nm compensates for turbidity.

3.8.2.3 Serum preparation

Blood samples from the rats were collected into centrifuge tubes and were left for about 45 minutes to clot. They were then centrifuged at 1000 rpm for 15 min after which the serum formed was collected into serum tubes with the aid of Pasteur pipettes. The serum samples were stored at -20°C in a

refrigerator in the research laboratory, Department of Nutrition and Food Science, University of Ghana, until needed for analysis.

3.8.2.4 Serum iron determination

The α, α - bipyridyl method was used (Osborne and Voogt, 1978).

The iron determination was done in triplicate using the following reagents and scheme:

- a. 10% solution of hydroxylamine in water.
- b. Acetate buffer solution of pH = 4.6
- c. 0.1% solution of α, α - bipyridyl in water.
- d. A standard iron solution prepared by dissolving ferrous ammonium sulphate in HCl and deionised water to give a final concentration of 10 μg per mL.

Six colorimeter tubes, each containing the following solution and reagents were set up as follows:

Table 3. Determination of serum iron concentrations of rats fed experimental diets

Tube No	Source of Iron	Weight of Iron (μg)	Hydroxylamine Solution(ml)	Acetate Buffer	Water (ml)	Dipyridyl (ml)	Conc ($\mu\text{/tub}$)
1	0.0ml std	0	1.0	2.5	4.5	2.0	0.0
2	1.0 "	10	1.0	2.5	3.5	2.0	10.0
3	2.0 "	20	1.0	2.5	2.5	2.0	20.0
4	3.0 "	30	1.0	2.5	1.5	2.0	30.0
5	4.0 "	30	1.0	2.5	0.5	2.0	40.0
6	2.0ml Digest	-	1.0	5.0	0.0	2.0	-

In each case, acetate buffer was added within few minutes of adding hydroxylamine hydrochloride. The contents of each tube were mixed using a vortex mixer. They were allowed to stand at room temperature, with occasional mixing, for 20 minutes for colour development. The Standard and the unknown mixtures were read at 500 nm using a Shimadzu spectrophotometer (P/N 204-000/0-02,uv-120-02, Japan) against a reagent blank (tube 1), which was used to standardize the instrument. A standard curve was plotted from the standard solutions containing 0.0, 10.0, 20.0, 30.0 and 40.0 µg of iron to obtain a linear regression equation for calculating the concentration of iron in the serum as follows:

$$\text{Iron concentration } (\mu\text{g}) = (\text{absorbance} \times 68.603) - 0.2105.$$

3.8.2.5. Packed cell volume

Blood from the rat was drawn into a micro-haematocrit tube and then centrifuged in a micro-haematocrit centrifuge (Gelman Hawksley Ltd, Sussex, U.K) at 1000 rpm for 10 min after which the % PCV was read using the Hawksley PCV reader. [Gelman Hawksley Ltd, Sussex, U.K]

3.8.3. Clinical Examination

The rats were examined daily for signs of poor growth, sore, hair losses, failure to develop normal pigmented incisors, loss of tonicity and other visible changes as well as activity and behavior. Daily observations were recorded.

3.8.4. Determination of iron in the feed and clay samples

The same method, α , α' - bipyridyl method, as for the serum iron was used but with a slight modification. In this case, aliquots of the wet digest solution for the Clay and feed were used (see Appendix 3).

3.8.5. Wet-ashing of the clay and diet aliquots

The method described by Pearson (1976) was used. Approximately 1g of the rat feed and 0.5g of the clay sample were weighed into separate 250ml beakers, 25ml of concentrated nitric acid was added to each sample and the beakers covered with watch glass. Each beaker with its contents was heated in a fume chamber for thirty minutes for the samples to digest.

After thirty minutes the pale yellow solution in each of the beakers was cooled and 2ml of perchloric acid were added. The contents were carefully mixed after which the digestion of the solutions continued until the solutions became almost colourless. After the digestion, the solutions were cooled and 30ml of distilled water was added to each solution and heated to boiling after which time each solution was filtered through # 44 Whatman filter paper into a 100ml volumetric flask. Each solution was then cooled and made up to 100ml mark with deionised distilled water.

3.8.6. Moisture determination

The air oven method described by Osborne and Voogt, (1990) was used. This was done in the laboratory so as to determine the moisture content of the clay

and the rat feed samples. This is to allow for calculations on dry weight basis. Approximately 0.5g of each sample was weighed into a clean dried moisture dish and placed into an air drought oven for 12 hrs. The moisture dishes were cooled in a desicator and weighed using an electronic digital weighing scale (G 160, Ohaus Electronic Balance). The moisture content determination was done in triplicate for each sample. The sample was quantitatively weighed into the moisture dish so that by subtracting the weight of dish, the sample weights could be obtained. The moisture contents of each sample was calculated using the formula below:

$$\% \text{ Moisture} = \frac{(W_1 - W_2) 100}{W_1}$$

Where:

W_1 = weight of sample before drying.

W_2 = weight of sample after drying.

3.9 Data presentation and analysis

Data analyses were computer based using Intercool STATA for windows 5.0 (STATA Corporation, Texas, USA). Graphical presentation and tabulations were done for better illustration of results. Differences between measured responses were studied using two-way ANOVA. Where differences existed, Dunnett's multiple comparison test was used to ascertain differences between the control group and all other groups. Multiple comparisons among all group

means were done with Tukey's adjustment. All findings were statistically tested for significance at $P < 0.05$.

3.10. Precautionary measures

The following precautions were taken.

- ◆ The rats were kept in the animal room and fed the experimental diets for 2 weeks to enable them acclimatize to the new environment.
- ◆ Movement to and from the animal room was minimized as much as possible to avoid disturbance of the rats.
- ◆ The room temperature was maintained between 25 - 28°C.
- ◆ It was ensured that lighting of the room followed the diurnal pattern.
- ◆ The use of air conditioner was regulated to maintain the usual odour of the rat room, which is important for rat studies.
- ◆ Deionised distilled water was used throughout the study to prevent contamination.
- ◆ All glassware and containers were acid washed.
- ◆ Analytical grade chemicals were used.
- ◆ Rats' weights were taken on the same day and time to offset any diurnal variation in weight of the test animals.
- ◆ The rats were kept in the animal room for 3 weeks to enable them acclimatize to the new environment.



Plate 1: Rats fed on experimental diets at baseline

CHAPTER FOUR

RESULTS

Table 4.1 Average daily dietary intakes and proportions of clay and feed in the experimental diets of the rats.

Diet	Average daily Intake of clay (g)	% of clay in the rat diet/d	Average intake of feed without clay(g)	Average daily intake of diet(g)
Control	0.00	0.00	21.00	21.00
Test Diet I	0.20	0.95	20.80	21.00
Test Diet II	1.35	6.43	19.65	21.00
Test Diet III	0.33	1.57	20.67	21.00

4.1. Levels of clay and feed and consumption of the experimental diets

Table 4.1 shows the clay content of the various experimental diets. There were no significant differences in the amount of diet consumed per day among the rats in the four different diet groups. On the average, each rat in each diet group consumed approximately 21g of diet.

The concentrations of iron in the feed and clay sample were determined to be 48.64mg/100g of feed and 1283.3mg iron per 100 gram of clay. Based on this analysis, the total iron content of each experimental diet was determined (Table 4.3). Intake of iron from the experimental diet and from the clay ranged from 10 to about 27 mg per day.

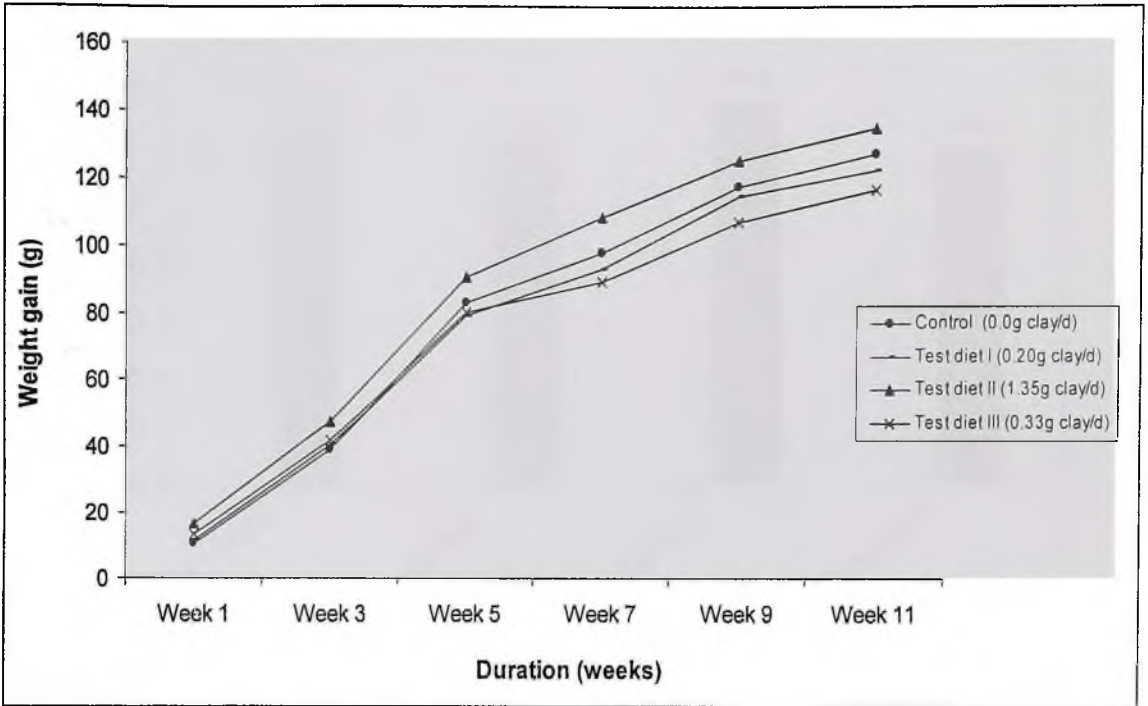
4.2 Characteristics of the experimental animals at the start of the study

In all, 32 young rats, age 3 weeks, were used for the study. Of these, 4 were randomly sampled for haemoglobin, PVC and body weight determination. This sub-sample of young rats had a mean (\pm SD) body weight of 39 ± 2.4 g (range, 32.0-51.3g), with mean haemoglobin concentration of 12.2 ± 0.6 g/dl (range, 11.5-12.8g/dl) and the mean PCV of 39.8 ± 2.5 % (range 38.3-43.5). These values were slightly below the normal for the adult rat (Appendix 2).

4.3. The growth rate of the experimental rats

The mean weekly body weight gain of the experimental rats in each diet group was not significantly different from the control (Figure 3). Similar observations were found for total body weight (Appendix 4) and growth in length (figure 5). Even though there was no significant differences in the rate of weight gain of the rats in the various diet groups and the control diet ($p>0.05$), the rats on test diet II, with the highest clay concentration, fared slightly better compared with the control group (Figure 3, Appendix 3).

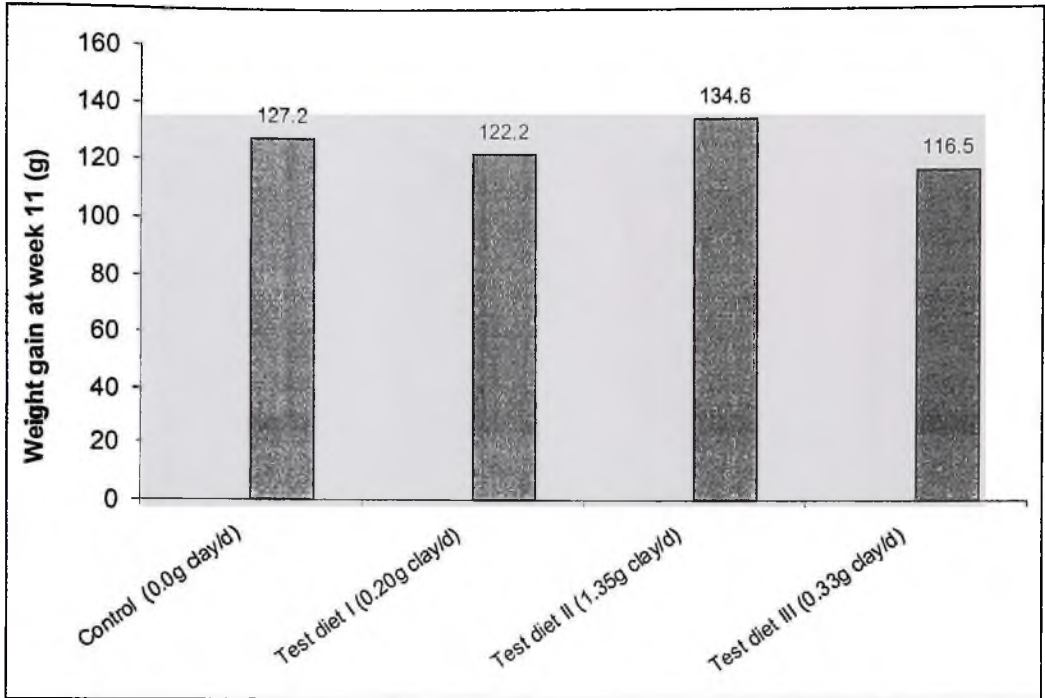
Figure 3. Mean weekly incremental weight gain of rats on experimental diets containing different levels of white clay^{1,2}



¹Weight gain was the difference in weight of the rats between the initial weight (week zero) and the index week.

²No significant differences in weight gain were observed among rat groups on the experimental diets, $P > 0.05$, Tukey's test

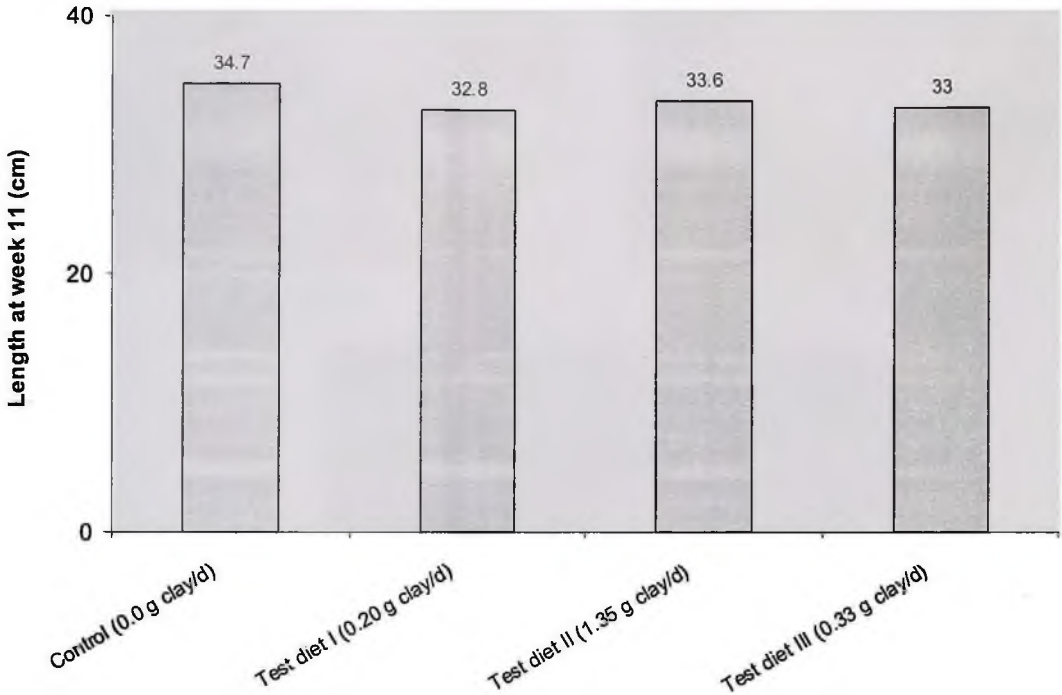
Figure 4: Total weight gain of rats fed different levels of white clay for 11 weeks^{1,2}



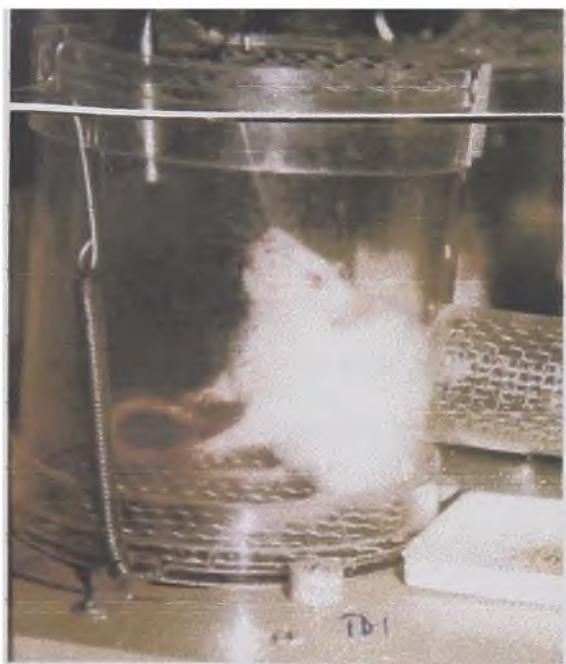
¹Total weight gain was the difference in weight of the rats between the initial weight (week zero) and the final week (week 11).

²No significant differences were observed between all diets, $P > 0.05$, Tukey's test

Figure 5: Growth in length of rats fed experimental diet containing different levels of white clay for 11 weeks¹



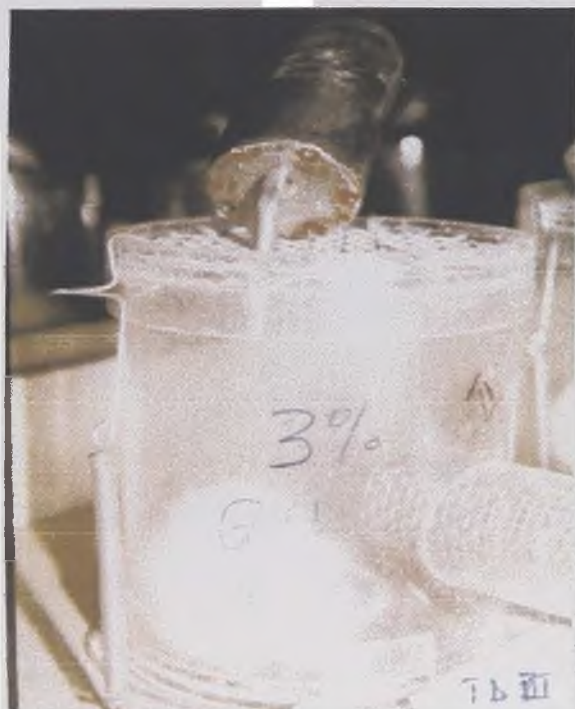
¹No significant differences were observed between all diets, $P > 0.05$, Tukey's test



A rat on TDI



A rat on TDII



A rat on TDIII

Plate 2: Rats fed on the clay diets for 10 weeks

4.4. Indicators of iron status

Table 4.2 shows the haemoglobin concentrations, PCV and serum iron Concentration of rats fed experimental diets containing different clay concentrations. All the rats on the three experimental diets had significantly higher haemoglobin concentration and PCV than the control group (Table 4.2).

The rats on test diet II, with the highest clay concentration, had significantly higher serum iron concentration than the rats on the other two test diets and the control group. However, the rats on the other two test diets had comparable serum iron concentration.

Table 4.2: Haemoglobin (Hb) concentration, PCV and serum iron concentration of rats fed experimental diets containing different levels of clay for 11 weeks compared with a control

Diet	Clay conc (g/d)	Hb conc (g/dl)	P-value ¹	PCV (%)	P-value ¹	Serum iron (mgFe/100 ml)	P-value ¹
Control	0.00	14.4 ± 0.42		43.0 ± 0.42		3.29 ± 0.35	
Test Diet I	0.20	17.6 ± 0.47	0.019	46.3 ± 0.45	0.016	2.91 ± 0.27	0.951
Test Diet II	1.35	17.4 ± 0.42	0.020	45.1 ± 0.36	0.045	9.33 ± 0.23	0.0001
Test Diet III	0.33	16.0 ± 0.26	0.041	44.7 ± 0.30	0.048	2.02 ± 0.20	0.108

¹All values less than 0.05 are significantly different from the control diet, Dunnett's test.

Table 4 .3: Concentration of iron (Fe) in the experimental diets consumed by rat per day

Diet	Clay conc. in diet (g)	Iron conc. from clay fed/d (mg)	Total conc. of iron in diet/d (mg)
Control	0.00	0.00	10.21
Test Diet I	0.20	2.57	12.69
Test Diet II	1.35	17.32	26.58
Test Diet III	0.33	4.23	14.28

4.5. Weights and appearance of essential body organs

Weights and appearance of each of the four essential organs; liver, kidney, heart and spleen of each of the test rats were studied (Table 4.4). There were no significant difference ($P > 0.05$) among the rats on the various diet groups in terms of the appearance and weights of the four essential body organs studied. The appearance of all the essential body organs was normal.

Table 4.4: Weights and appearance of essential body organs of rats fed experimental diets containing different clay concentrations¹

Diet	Control	Test diet I	Test diet II	Test diet III
Weight (\pm SD)				
Liver (g)	6.3 \pm 0.61	6.6 \pm 1.44	6.3 \pm 0.85	5.7 \pm 1.25
Spleen (g)	0.5 \pm 0.20	0.4 \pm 0.08	0.4 \pm 0.08	0.4 \pm 0.10
Kidney (g)	1.2 \pm 0.16	1.5 \pm 0.14	1.3 \pm 0.08	1.1 \pm 0.17
Heart (g)	0.7 \pm 0.07	0.7 \pm 0.12	0.7 \pm 0.13	0.6 \pm 0.16
Length at week 11 (\pm SD) (cm)	34.7 \pm 3.8	32.8 \pm 1.2	33.6 \pm 2.3	33.0 \pm 1.0

¹No significant differences were observed between all diets, $P>0.05$, Dunnett's test

4.6. Clinical symptoms

Clinical examination was performed on the rats every day for the 11 weeks study duration. No discernible clinical symptoms were observed on any of the rats in the experiment. There were no body lesions and no signs of hair loss. There was normal development of pigmented incisors in each rat.

CHAPTER FIVE

DISCUSSIONS

5.1 Iron Status

Consumption of the experimental diets containing different concentrations of clay was associated with significant increases in the selected indicators of iron status in the experimental rats. The rats on the clay diets had significantly higher haemoglobin and serum iron concentrations than those on the control diet ($P < 0.05$). This might have contributed to the higher PCV values obtained for the group of rats on the clay diets.

Laboratory analysis of the clay sample revealed that it contains a very high concentration of iron (1283.3mgFe/100g clay). This result agrees with the one by Koduah (2000), which revealed clay as a potential source of iron for clay eaters. The amount added to each experimental diet increased the iron content of the diet. The clay contributed 2.57mg iron/d to test diet I containing 0.20g clay/day; 17.32mg iron to test diet II and 4.23mg of iron to test diet III containing 0.33g clay/d. This implies that each diet contained more than 3-fold of the recommended dietary iron requirement for rats; 3.5mg/kg diet (National Research Council, 1978).

5.2 Haemoglobin concentrations

The rats on the clay diets had slightly higher haemoglobin concentrations compared with the controls. This observation is in line with the reports by Talkington et al, (1971); Bronstein and Dollar, (1974); Coltman, (1969) and

Latham, (1997) which indicate that clay ingestion increases the haemoglobin level and apparently reduces or eliminate anaemia. On the contrary, Tayie and Lartey (1999) however, reported that eating of clay is associated with low haemoglobin levels.

Reports by Rainville, (1998); Gibbs and Seitchick, (1980) show a negative correlation with the haematological results obtained in this study. The low haemoglobin level reported by Tayie and Lartey, (1999); Rainville, (1998) and Gibbs and Seitchik, (1980) for eaters of clay may be due to confounders or limitations. In fact, the pregnant women might be anaemic and had very low haemoglobin levels before the study.

The type of diet, which is consumed along with the clay, may also contribute to the absorption of iron. Diets containing vitamin C and organic acids will aid the absorption of iron. Conditions of low iron status exist during pregnancy when a pregnant woman's iron requirement increases to about 300mg/100g (Williams, 1993). This amount of iron cannot be supplied by the average diet; even though the clay may provide some of the required iron, it may not be enough to meet the requirement for pregnancy. Other reasons have also been identified as non-heme iron sources, such as iron from clay, which is only about 5-10% absorbable (Barasi, 1997) but absorption may be more in pregnancy. Even though the clay may have a higher mineral content, the actual amount of iron absorbed into the body may be small. The clay could provide excess amounts of minerals that competitively inhibit the absorption of iron and other nutrients required for haematopoiesis (Tayie and Lartey, 1999).

5.3 Growth

The concentration of iron and/or clay in the diet had no significant effect on food intake and hence growth of the rats. There were no visible signs of growth retardation. All the rats in the various diet groups grew normally following the normal growth curve, which indicates that there were no statistically significant differences in their growth rates.

Since there were no significant differences in growth among the rats on the clay diets compared to controls, it is therefore suggested that clay ingestion might not affect the birth of an infant whose mother eats clay. These confirm the reports of Tayie and Lartey, (1999) that the observed levels of clay intake by pregnant women in Ghana did not significantly affect infant birth-weight. The findings from the present study also agree with the report of Rainville, (1998) that pica practices have no effect on pregnancy outcomes. Likewise, there were no differences in the lengths of the rats on the various experimental diets after 11 weeks of study. They were almost of the same length.

5.4 Clinical signs and effects on body organs

The study did not identify any clinical symptoms suggestive of nutrient deficiencies on rats with and without clay in the diet. Symptoms of anaemia such as poor growth, presence of sore, hair losses and loss of tonicity were absent. There were no visible changes in their activities and behaviour. It appears that the clay consumed had no effect on their iron status; hence, they were not anaemic.

There were no group differences ($P>0.05$) in the weights of the essential body organs (kidney, liver, heart and spleen) of the rats on the various diet groups. Hence, the essential organs of the rats did not differ significantly in appearance, size and weight from those of rats on normal diet (without clay). The results suggest that the clay ingested by the rats did not have any notable effect on the weights and appearance of these essential organs.

From this study, there was no evidence that clay consumption induces anaemia in rats. Rats fed different levels of clay had significantly higher haemoglobin concentration than rats on a no clay diet.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

From the results of this study, the following conclusions can be drawn:

1. Clay ingestion resulted in significant increase in haemoglobin concentration in the rats.
2. Clay ingestion resulted in significant increase in serum iron concentration in the rats when the amount of clay in the diet was above 5 %.
3. Clay ingestion resulted in significant increase in PCV in the rats.
4. Clay ingestion did not affect the sizes of essential body organs; kidney, liver, heart and spleen.
5. Growth and weight gain was not affected significantly by clay ingestion.
6. Rats on clay diet, at the level fed and the stated duration, do not show any outward clinical symptoms.
7. Ingestion of clay did not associate with the anaemia indicators studied.

6.2 RECOMMENDATIONS FOR FUTURE WORK.

1. Human volunteers should be used to validate this study.
2. A nationwide study of clay pica should be conducted.

3. Further studies must be carried out on the effects of clay ingestion on growth using longer duration.
4. Effects of clay ingestion on the gastro-intestinal tract should be investigated.

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APPENDIX 1.

The composition of the rat feed

Protein (CP).....	15% - 16%
Energy (ME).....	2800 kcal/kg
Crude Fibre.....	3.5%
Calcium.....	1.00%
Phosphorus.....	0.40%
Lysine.....	0.65%
Salt.....	0.40%

Net Wt. 45 kg

(Source: Kosher Feed Mills Ltd, Osu – Acca)

APPENDIX 2.

Rat Physiological data

<u>Index</u>	<u>Value</u>
Breeding life (yr)	1
Breeding Season	Anytime
Gestation Period (Days)	21
Duration of Estrus (Days)	4-5
Litter Size	6-9
Weaning Age (Weeks)	3-4
Breeding Age (Months)	2-3
Birth-weights (gm)	5-6
Weight at weaning (gm)	40-50
Body temperature (° C)	38.2
Blood Pressure (mm Hg)	130/90
Blood Volume (ml/kg)	65
Clot Time (sec.)	60
RBC sedimentation. Rate (mm/hr)	1.6
Haematocrit (% RBC)	46
Haemoglobin (gm/100 ml)	14.8
Water consumption (ml)	35
Environmental Temperature Range (° F)	65-75 45-50
Relative Humidity (%)	0.30 – 0.60
Adult Body Weight (kg)	3
Life Span (yrs)	4
Maximum Life Span (yrs)	

(Source: Grindeland, 1996).

Appendix 3. Mean weekly incremental weight gain of rats on experimental diets containing different levels of white clay

Diet group	Clay intake (g/d)	Mean weight gain (g)					
		Week 1	Week 3	Week 5	Week 7	Week 9	Week 11
Control	0.00	10.5	39.1	82.9	97.9	117.5	127.2
Test diet I	0.20	11.3	40.8	79.4	93.2	114.6	122.2
Test diet II	1.35	16.5	47.9	90.6	108.3	125	134.6
Test diet III	0.33	13.3	41.9	80.1	89.1	107.2	116.5

Appendix 4. Mean weekly body weights of rats fed experimental diets containing different clay concentrations

Diet	Clay intake (g/d)	Mean weekly body weights (g)											
		0	1	2	3	4	5	6	7	8	9	10	11
Control	0.00	41.7	52.2	62.7	80.8	101.1	124.6	130.5	139.6	150.5	159.2	167.0	168.9
TD I	0.20	36.0	47.3	58.8	76.8	97.4	115.4	121.5	129.2	141.7	150.6	165.5	158.2
TD II	1.35	38.5	55.0	65.0	86.4	111.9	129.1	138.1	146.8	157.7	163.5	173.1	173.1
TD III	0.33	40.1	53.4	65.2	82.0	107.0	120.2	125.8	129.2	142.0	147.3	156.6	156.6